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Synergistic effects of resveratrol (free and inclusion complex) and sulfamethoxazole-trimetropim treatment on pathology, oxidant/antioxidant status and behavior of mice infected with *Toxoplasma gondii*

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ABSTRACT

This study aimed to investigate the synergistic effects of resveratrol and sulfamethoxazole-trimethoprim (ST) on the treatment of mice experimentally infected by *Toxoplasma gondii* during the chronic phase of the disease considering infection, behavior, and oxidative/antioxidants profile aspects. For the study, 60 mice were initially divided into two groups: uninfected (n = 24) and infected by *T. gondii* (n = 36). These two groups were later subdivided into other groups and treated with resveratrol (free and inclusion complex containing resveratrol) alone and co-administered with ST: groups A to D were composed by healthy mice and groups E to J were consisted of animals infected by *T. gondii* (VEG strain). Treatments began 20 days post-infection for 10 consecutive days with oral doses of 0.5 mg kg^{-1} of ST (groups B and F), 100 mg kg^{-1} of free resveratrol (groups C and G) and inclusion complex of resveratrol (nanoparticles containing resveratrol) (groups D and H), and lastly an co-administration of both drugs (groups I and J). Behavioral tests (memory, anxiety and locomotion) were performed after treatment. Liver and brain fragments were collected to evaluate pathological changes, brain cysts counts, as well as oxidant and antioxidant levels. A reduction on the number of cysts in the brain of animals treated with both drugs combined was observed; there was also reduced number of lesions on both organs. This drug combined effect was also able to reduce oxidative and increase antioxidant levels in infected mice, which might be interpreted as a resveratrol protective effect. In addition, the combination of ST and resveratrol was able to prevent behavioral changes in infected mice. Therefore, the use of co-administration drugs enhances the therapeutic effect acting on a synergic way, reducing the oxidizing effects of the chemical treatment for toxoplasmosis. In addition, resveratrol in inclusion complex when co-administered with ST showed an improved therapeutic effect of ST reducing oxidative damage, liver damage and the number of cysts in the brain of *T. gondii* infected mice.

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1. Introduction

Toxoplasma gondii is a coccidian parasite belonging to the phylum Apicomplexa, which includes the largest and most important group of obligate parasites [1]. It has a great capacity to parasitize various types of cells and infects almost any warm-blooded animal [2]. Humans and other animals may become infected by *T. gondii* by the ingestion of sporulated oocysts shed in cat feces, which are the definitive hosts [2]. Humans can also become infected by ingesting raw or undercooked meat from infected animals.

It is estimated that about one third of the world population is seropositive for toxoplasmosis [3]. In the acute phase, tachyzoites rapidly invade nucleated cells and begin to replicate. In addition, the parasite may migrate into tissues where they may form cysts containing bradyzoites [4]. This multiplication favors infection at various sites of the body, such as the central nervous system.

Currently, sulfadiazine is the drug of choice to treat toxoplasmosis in humans. However, this drug has some toxicity and causes side effects, despite being widely used in the treatment of toxoplasmosis as reported by researchers [5–8]. Studies shows that when this compound or similar are associated with antioxidants, there is an increase in therapeutic efficacy and a reduction cell injury and cysts number when compared to animals treated only with chemotherapy [9,10]. Based on these considerations, resveratrol was tested in this study as a probable therapeutic drug against toxoplasmosis considering parasitemia, behavior, oxidant/antioxidant status, and pathological findings being a natural drug that could reduce the side effects of ST.

Resveratrol (3,4′,5-trihydroxystilbene) is a polyphenol with high antioxidant potential, naturally found in wine, peanuts, grapes, and other fruits [11,12]. Numerous studies have investigated the attributes of this biological compound which mainly include antioxidant and anti-inflammatory activities, platelet anti-aggregator effects, anti-atherogenic property, growth inhibition activity, and immunomodulation [12]. Despite being a powerful antioxidant, resveratrol is easily destroyed by acids and enzymes present in the digestive tract [13]. Therefore, there is a need to develop more effective methods to deliver and metabolize this compound. Due to these implications, the inclusion complex of resveratrol was chose in this study, since it has better therapeutic effect when interacting with body tissues on a specific manner with enhanced absorption and slower release [14]. Based on this information one question arises: does the combination of resveratrol with sulfamethoxazole-trimethoprim lead to clinical, and biochemical improvement? Therefore, this study aimed to assess the synergistic effects of resveratrol (free and inclusion complex of resveratrol) co-administered with sulfamethoxazole-trimethoprim in the treatment of mice experimentally infected by *T. gondii* during the chronic phase of infection considering the aspects of behavior and oxidative profile.

2. Materials and methods

2.1. Toxoplasma gondii strain and inoculum preparation

T. gondii tachyzoites from VEG strain (type III) previously kept in liquid nitrogen was inoculated in one Swiss male mice, this procedure was done in order to reactivate parasite's virulence. Thirty days later, brain of infected mice was homogenized (in saline solution), the cysts containing bradyzoites was collected and inoculated orally in the experimental mice (n = 36) in order to simulate the chronic phase of toxoplasmosis. The animals used in this study are from Universidade do Estado de Santa Catarina (UDESC).

2.2. Animal model

Sixty Swiss male mice with a mean age of 60 days weighing 25 ± 5 g were kept in boxes with five animals each, under a 12 h light/dark cycle with controlled temperature and humidity (25 °C, 70% respectively). The animals went through an adaptation period of 10 days and were fed with commercial feed and water *ad libitum*.

2.3. Experimental design

Firstly, 60 animals were divided into groups: group A-D (n = 24) consisted of healthy uninfected mice, and groups E-J (n = 36) with animals infected orally with 50 cysts containing bradyzoites of a cystogenic strain (VEG) of *T. gondii*. After 20 days post-infection (PI) have the treatments began, and the detailed experimental design is shown in Table 1.

Treatments started 20 days PI with sulfamethoxazole-trimethoprim (ST), free and inclusion complex containing resveratrol or combined effect of drugs orally administered for 10 consecutive days at a dose of 0.5 mg kg^{-1} for ST; and 100 mg kg^{-1} for free and inclusion complex of resveratrol (Table 1).

2.4. Characterization of the resveratrol and inclusion complex

Resveratrol ($C_{14}H_{12}O_3$; molecular weight ¼ 228.25 g mol^{-1} ; purity >98%) and 2-hydroxypropyl-β-cyclodextrin (HPβCD) were obtained from Sigma Aldrich. A mixture containing 0.1 mM of HPβCD, water (8 mL at 40 °C), and an equimolar amount of resveratrol (0.1 mM) was prepared by vigorous stirring for 1 min in an ULTRA-TURRAX High-Speed Homogenizer T 25 (IKA, Reino Unido) at 3.200 rpm. RSV, HPβCD, and the complex spectra were collected using an FT-IR in a spectral region between 4000 and 450 cm^{-1}. All of these procedures were carried out in the Chemistry Laboratory of the Federal University of Santa Maria (UFSM).

2.5. Behavioral tests

2.5.1. Elevated plus-maze (EPM)

This test has been widely used in rodents to measure anxiety [15]. The apparatus consist of two elevated (26 cm high) and open arms (16 × 5 cm) positioned opposite to one another and separated by a central platform (5 × 5 cm), and two arms of the same dimension, but enclosed by walls (16 × 5 × 10 cm) forming a cross. During a 5 min test period, the number of entries either to the open or closed arms, plus the time spent in the open arms were recorded. The anxiolytic effectiveness was illustrated by a significant statistical augmentation of parameters in open arms (time and/or entries) [16]. The EPM training was performed on day 30th and tested on 31st.

2.5.2. Passive avoidance task

Long-term memory (LTM) was investigated using passive avoidance task according to the method of Sakaguchi et al. [17] with modifications in the intensity and exposure time for the electric shock. During the training session, each mouse was placed on the platform. An electric shock (0.5 mA) was delivered for 2 s every time the animal completely stepped on the grid floor. LTM training was performed on day 30th and tested on 31st.

2.5.3. Open-field test

The open-field testing apparatus was made of square plywood (40 cm × 40 cm) surrounded by walls of 30 cm high containing marks on the floor made by tape markers, thus, dividing each square into 9 squares (3 rows of 3). Each animal was placed individually at the center of the apparatus and its movements (number